Identification of amino-acid residues on intercellular adhesion molecule-4 that mediate adhesion to its α_v integrin ligands

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Intercellular adhesion molecule-4 (ICAM-4) is expressed chiefly on erythroid cells and is the glycoprotein that carries the LW blood group antigens. We recently reported that ICAM-4 binds hemopoietic (HEL) and nonhemopoietic (HT1080) cell lines and that the cellular ligands for ICAM-4 are the $\alpha_4\beta_1$ integrin and α_v integrins (most notably avb1 and avb5) respectively (Spring et al, Blood 2001 98: 458-466). In order to elucidate the structural basis of integrin-ICAM-4 interaction we have carried out a systematic analysis of the role of surface-exposed residues, by site-directed mutagenesis, using a molecular model of ICAM-4 derived from the crystal structure of ICAM-2. The model presents ICAM-4 as two Ig-like domains; domain 1 being N-terminal of the membrane anchored domain 2. Each domain has two faces (or sides); the ABE and the CC'FG faces. Mutagenesis of ICAM-4 has revealed that a number of single amino acid changes affect α_v integrin-mediated adhesion to ICAM-4. Two mutations, T162V and N160A, are "super adhesive", increasing the level of adhesion between ICAM-4 and HT1080 cells. Size analysis by SDS-PAGE revealed that the T162V and N160A mutants are slightly smaller than native ICAM-4, suggesting that these two mutations prevent the N-glycosylation of asparagine 160. Mutations to ten other amino acid residues in ICAM-4 lead to a reduction in adhesion to HT1080 cells. Most of these residues are found at the top of domain 1 in a run of three amino-acids on the A strand (F18A, W19A and V20T) and five of a run of six amino-acids on the G strand (R92E, A94L, T95V, S96A and R97E). These residues identify a footprint on the ICAM-4 molecule that straddles the edges of both the ABE and C C'FG face of domain 1. Two other mutations lead to a reduction in adhesion to HT1080 cells, one in the ABE face of domain 1 (W66A) and the other in the ABE face of domain 2 (K118E). Our findings suggest that contact between ICAM-4 and α_v integrins involves a large extent of the surface of ICAM-4, with the footprint on domain 1 being a critical site in mediating this interaction. Integrin-mediated α_v adhesion to ICAM-4 may play a role in the formation of erythroblastic islands in the bone marrow (during erythropoiesis) and in the abnormal adhesion of red cells to activated endothelium in sickle cell disease. These studies inform our understanding of the structural basis of these interactions and provide a foundation for further investigation of their biological significance.

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